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ACUTE AND LONG-TERM EFFECTS OF ISOFLURANE AND SEVOFLURANE ANAESTHESIA IN LABORATORY MICE

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1. ABSTRACT

Isoflurane is to date the most common volatile anaesthetic in laboratory rodents whereas the modern sevoflurane is usual for inhalation anaesthesia in human medicine. In this study it was aimed to characterize and compare the clinical properties and safety of both anaesthetics for anaesthetizing mice.

In an approach that mirrors the laboratory routine (spontaneous breathing, gas supply via nose mask, preventing hypothermia by a warming mat) a 50-minutes anaesthesia was performed. Anaesthetics were administered at standardized dosages of 1.5 X minimum alveolar concentrations (1.85% for isoflurane, 3.25% for sevoflurane) in 100% oxygen.

Induction and recovery from anaesthesia proceeded quickly, within 1 and 2 minutes respectively. During anaesthesia, all reflex testing were negative and no serious impairment of vital functions was found; all animals survived. Most prominent side effect during anaesthesia was the respiratory depression with marked decrease of respiration rate, hypercapnia and acidosis. Under anaesthesia, heart rate and core body temperature remained stable and within normal range, but were significantly increased for 12 hours after anaesthesia. Locomotor activity, food and water consumption and body weight progression showed no abnormalities after anaesthesia. No relevant differences between the two anaesthetics were found.

In conclusion, both anaesthetics equally provide high safety margin with acceptable side effects and are therefore recommended for anaesthesia in laboratory mice.

2. THEORETICAL BACKGROUND AND ANAESTHESIA MILE STONES

2.1. General anaesthesia

Anaesthesia (from Greek αν-, an-, "without"; and αἴσθησις, aisthēsis, "sensation") is defined as the absence or abolition of sensation. This term should be differentiated from analgesia which is defined as the absence or abolition of pain. General anaesthesia involves rendering a patient unconscious whereas local anaesthesia (or more correctly local analgesia) is aimed at blocking conduction of nerves to the operative site. In order to provide safe as well as adequate general anaesthesia, the anaesthesiologist must combine the need for unconsciousness, the need for analgesia, and the need for muscle relaxation to provide the best operative conditions for the surgeon.

There are many ways to design an anaesthetic plan to meet the requirements for general anaesthesia: muscle relaxation appropriate for the procedure, unconsciousness, and analgesia. Both volatile and injection anaesthetics can meet those requirements.

Four main stages of general anaesthesia are recognized regardless of the method in which the anaesthesia is delivered. These stages are based upon the patient's body movements, respiratory rhythm, oculomotor reflexes, and muscle tone.

In general, a patient in stage one is conscious and rational, however the perception of pain is diminished. Stage one is commonly termed the analgesia stage.

Stage two, the delirium stage, is marked by the patient becoming unconscious, however the body responds reflexively and irrationally to stimuli. Breath holding may be present and can result in hypoxia, however tone is maintained in pharyngeal muscles and a patient can maintain and protect it's own airway. Pupils generally become dilated.

Stage three, the surgical anaesthesia stage, is characterized by increasing degrees of muscular relaxation. Protective pharyngotracheal reflexes are absent and the patient is unable to protect the airway.

Stage four is medullary depression. This stage is characterized by cardiovascular and respiratory collapse due to depression of the cardiovascular and respiratory centers in the brain stem.

2.2. Types of anaesthesia

2.2.1. Injection anaesthesia

In the past, injection anaesthesia has played a major role in work with laboratory rodents. However, it's use has been confided by several facts, like large differences in sensitivity and dosages between species and strains, the fact that it is virtually uncontrollable once injected and that very long recovery periods occur in some cases. Moreover the recommended dosages for specific species or strains vary considerably (up to a factor 10) between different protocols [1], opening a door for various unexpected adverse effects, insufficient anaesthesia or high death rates.

This situation led, in the past years, to a steady increase in the use of inhalation anaesthesia in laboratory animal routine [1, 2].

2.2.2. Inhalation anaesthesia

Inhalation anaesthesia refers to the delivery of anaesthetic vapours into the body via the respiratory tract and through uptake and distribution, a portion of the anaesthetic agent is presented to the nervous system, resulting in the absence of sensation. Induction and recovery are quick, depth of anaesthesia is good controllable, and the anaesthesia can be adapted to any individual need. Thanks to those characteristics it is becoming more and more present in laboratory rodents

2.3. Minimum alveolar concentration

The idea of minimum alveolar concentration was introduced in 1963 by Eger and Merkel, who described a technique for establishing a minimal concentration of anaesthetic required to prevent gross muscular movement in response to a painful stimulus [3].

Minimum alveolar concentration is a concept used only for volatile anaesthetics. It is the equivalent of effective dose 50 (ED_{50}) for other, non volatile drugs. In simple terms, it is defined as the concentration of the vapour in the lungs that is needed to prevent movement (motor response) in 50% of subjects in response to a surgical (pain) stimulus. This measurement is done at steady state, by providing a constant alveolar concentration for 15 minutes, allowing for an equilibration between the gasses in the alveoli, the blood and the brain to take place. It is a clinically useful operational definition, allowing easy comparisons between the potency of different anaesthetic agents and featuring impressive stability [4, 5].

Thus, although the term minimum alveolar concentration was used by Eger in the original paper, it is actually a median value.

2.4. Surgical tolerance

Unconsciousness, analgesia and blockade of somatic motor response to noxious stimulation are the most important goals of general anaesthesia [4]. Volatile anaesthetics such as isoflurane, sevoflurane and halotane are frequently used in modern clinical and laboratory practice, to provide hypnosis (unconsciousness) and a loss of responses to noxious-evoked stimuli (immobility).

Surgical tolerance is a term that refers to a depth of anaesthesia reached when the subject does not react with a motor response to a noxious stimulus in form of a skin incision.

By providing anaesthetics at concentrations of 1.5 X minimum alveolar concentration, it is generally postulated, that 99.9% of animals do not react to painful stimuli [6, 7], i.e. that they have reached surgical tolerance.

2.5. Volatile anaesthetics

The volatile anaesthetics are a class of general anaesthetic drugs. Most of them share the property of being liquid at room temperature, but evaporating easily for administration by inhalation (e.g. halothane, isoflurane, sevoflurane). The fundamental mechanisms by which these inhalational agents induce anaesthesia are similar, although not completely understood [8].

There are quite a few inhalant anaesthetics available for use in human and veterinary medicine (nitrus oxide, halothane, enflurane, desflurane etc.). However, confined by frames of this work, only the two agents used in our study will be presented below.

2.5.1. Isoflurane

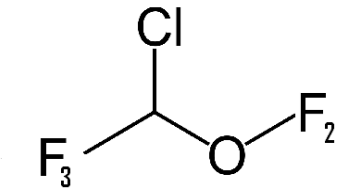

Figure 1 Chemical structure of isoflurane (2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane) [9].

Isoflurane (2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane) is a halogenated ether used for inhalational anaesthesia (Figure 1). Its use in human medicine has declined during the last decade, being replaced mainly with sevoflurane, desflurane and the intravenous anaesthetic propofol. Nevertheless isoflurane is still frequently used for veterinary anaesthesia especially in horses, dogs and cats [10].

It has low blood-gas partition coefficient (Table 1), which enables quick induction and recovery abilities. It is virtually not metabolized (metabolic rate around 0.2%) [10], and it is reported to have weak analgesic effect in humans.

2.5.2. Sevoflurane

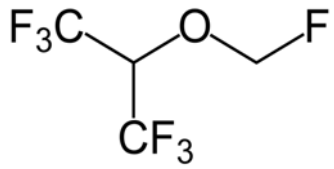

Figure 2 Chemical structure of sevoflurane (2,2,2-trifluoro-1-[trifluoromethyl]ethyl fluoromethyl ether) [9].

Sevoflurane (2,2,2-trifluoro-1-[trifluoromethyl]ethyl fluoromethyl ether), also called fluoromethyl hexafluoroisopropyl ether, is a sweet-smelling, non-flammable, highly fluorinated methyl isopropyl ether used for induction and maintenance of general anaesthesia (Figure 2).

Like isoflurane, sevoflurane has a low blood-gas partition coefficient (Table 1), but the fact that is less irritant to mucosa and that it has some analgesic effect made it very common in human medicine. It is still rarely used in veterinary practice.

	Sevoflurane	Isoflurane
Molecular weight	200.005 g/mol	184.5 g/mol
Boiling point (at 1 atm.):	58.6 °C	48.5 °C
Density:	1.517–1.522 g/mL	1.496 g/mL
MAC (Human) :	2 vol %	1.15 vol %
Vapor pressure (at 20°C):	157 mmHg (20.9 kPa)	238 mmHg (31.7 kPa)
Vapor pressure (at 25°C):	197 mmHg (26.3 kPa)	295 mmHg (39.3 kPa)
Vapor pressure (at 35°C):	317 mmHg (42.3 kPa)	450 mmHG (60.0 kPa)
Blood:Gas partition coefficient:	0.68	1.4
Oil:Gas partition coefficient:	47	98

Table 1 Overview of physical characteristics of the two volatile anaesthetics.

2.6. Unwanted effects of anaesthesia

The ideal anaesthetic agent would produce reversible unconsciousness, analgesia and muscle relaxation, with no effect on other physiological functions. Unfortunately, no anaesthetic drug meets this standard and they all affect other body systems to some extent. The most serious side effect of anaesthetic drugs is depression of respiration and the cardiovascular system. There are two reasons why these side effects are important:

1) They occur at "therapeutic" doses, i.e. there is significant depression at the doses of anaesthetic needed to produce anaesthesia.

2) Severe depression of either respiration or the cardiovascular system is life-threatening.

Although the pharmacodynamic effects of anaesthetics vary, the mechanisms by which they cause anaesthesia emergencies are usually the same: excessive hypotension, bradycardia, arrhythmias, myocardial depression, vasodilatation or vasoconstriction, hypoventilation followed by hypoxemia [11].

2.6.1. Effects on respiration and acid-base homeostasis

By depressing respiratory centres in the brain stem, all anaesthetic agents show adverse effects upon respiration. Diminished respiration rate or tidal volume, together with arrhythmic breathing or even apnoe episodes, are very common during anaesthesia and have obvious and detrimental effects on oxygen (O_2) tissue supply as well as on subsequent carbon dioxide (CO_2) elimination.

Arterial blood gas analysis is the gold standard for assessing respiratory function. The gas exchange capability of the lung can be directly measured. The three main measurements (partial pressure of oxygen (pO_2), partial pressure of carbon dioxyde (pCO_2) and pH) made by the blood gas analyzer are used together to obtain a detailed picture of the state of respiratory system [11].

2.6.1.1. Partial pressure of carbon dioxide

It is a measure of ventilatory status of the patient and normally ranges between 35 mmHg and 45 mmHg in most mammalian species. In mice these values are slightly lower and average around 25 mmHg [1].

Hypercapnia (increase in pCO2) may be caused by hypoventilation or by dead space rebreathing [11].

2.6.1.2. Partial pressure of oxygen

The pO_2 is a measure of the oxygenating efficiency of the lungs. The pO_2 measures the tension of oxygen dissolved in physical solution in the blood plasma, irrespective of the haemoglobin concentration. Haemoglobin saturation measures the percentage saturation of haemoglobin with oxygen and is related to the pO_2 by a sigmoid curve [12].

2.6.1.3. Acid-base homeostasis

The pH of the body must be maintained within a narrow range. Most body systems function optimally at intracellular pH of near 7.4. As the pH changes (either higher or lower), enzymes may cease to function, nerve and muscle activity weakens, and finally all metabolic activity becomes deranged.

2.6.1.4. Carbonic acid-bicarbonate buffering

The carbonic acid bicarbonate system is a classic chemical buffer. In addition, the body has the ability to eliminate chemicals from either end of the chemical reaction to maintain the pH. In the case of bicarbonate, the chemical reaction is

$$H+ \quad + \quad HCO_3- \quad \leftrightarrow \quad H_2CO_3 \quad \leftrightarrow \quad H_2O \quad + \quad CO_2$$

This buffering system is very effective because of the ability to convert carbonic acid to carbon dioxide (through the enzyme carbonic anhydrase) then remove CO_2 from the body through respiration. For example, adding enough acid to lower the serum bicarbonate by half would normally drop the pH from 7.4 to 6.0, but instead all the extra H_2CO_3 is removed by conversion to CO_2. The drop in pH stimulates extra respirations so CO_2 (and subsequently more H_2CO_3) is removed. The pH therefore falls only to 7.3 or 7.2.

On the other side of the equation, excess acid or excess alkali can be removed through the kidneys.

Changes in carbonic acid concentration occur rapidly (within seconds) in response to hypo- or hyperventilation. On the other hand, changes in bicarbonate require hours or days through the relatively slow process of elimination by the kidney.

The ratio of bicarbonate to carbonic acid determines the pH of the blood. Normally the ratio is about 20 parts bicarbonate to 1 part carbonic acid.

This relationship is described in the Henderson-Hasselbach equation:

$$pH = pK + \log (HCO_3-/H_2CO_3)$$

Thereby, pK is the dissociation constant of the buffer and is 6.10 at body temperature. The change in pK with temperature is the reason why pH determinations must be adjusted for patients with abnormal temperatures.

2.6.1.5. Respiratory acidosis

Build-up of carbon dioxide occurs when ventilation is inadequate. This is usually due to absence of adequate respiratory effort such as when central control of respiration

is depressed due to anaesthetics, narcotics or barbiturates. When respiration ceases due to cardiac arrest, of course, respiratory acidosis is an immediate result.

Respiratory acidosis can also result when obstruction of air motion leads to carbon dioxide build-up [12].

2.6.1.6. Compensation of respiratory acidosis

Whereas respiratory changes can occur within seconds or minutes, metabolic changes take hours to days. Compensation for respiratory acidosis must occur through elimination of acid through the kidney. Only in chronic respiratory problems, such as severe obstructive airway disease, will compensation be seen.

2.6.2. Effects on cardio-vascular system

Anaesthetic agents usually have impact on the function of heart and circulation. Peripheral vasodilatation and decrease of heart rate, cardiac output, and blood pressure are such adverse effects of anaesthesia which can lead to hypotension, possible heart arrest and finally death of the animal [13]. Mild hypotension is mostly tolerated by healthy animals but prolonged serious hypotension (mean arterial pressure <60 mmHg) produces renal shutdown. From this, the animal can go into renal failure and may die after anaesthesia. Therefore, it is mandatory to control the impairment of heart functions and circulation during anaesthesia to prevent irreversible harm or sudden death during and after an anaesthesia.

2.6.3. Effects on body temperature regulation

General anaesthetics interfere with temperature regulation both by depressing the hypothalamic centres and by suppressing heat generation mechanisms such as shivering. This adverse effect of anaesthesia falls in weight by laboratory rodents even more than in other species. Small rodents, because of their relatively low body mass, have a high body surface area-to-mass ratio. This high ratio combined with suppressed thermoregulatory mechanisms allows for rapid escape of body heat in small rodents and consequently they are highly susceptible to hypothermia during anaesthetic events [14]. Small mammals can experience a decrease of 4-10°C in core body temperature during only 15-20 min of general anaesthesia, resulting in marked hypothermia [14, 15].

Mild to moderate hypothermia does not kill animals but leads to other problems, the most important being cardiac arrhythmias, increased susceptibility to infection, prolonged anaesthetic recovery time, and decreased minimum alveolar concentration values for inhalant anaesthetics, resulting in increased potential for anaesthetic toxicity [14, 16].

In order to control body temperature and maintain it at the normal level we need to have a means of measuring temperature and correcting it (e.g. heating blankets, insulation etc.) if necessary.

2.7. Monitoring during anaesthesia and post-anaesthetic recovery

The purpose of anaesthesia is to provide a reversible status of hypnosis, muscle relaxation, analgesia, and diminished reflexes while the adverse effects and the overall risk for the patient are minimized. When we consider the main side effects of anaesthesia (hypothermia, cardiovascular and respiratory depression) the argument for monitoring becomes very compelling. In the research community, even more than in the clinic, the question of post-anaesthetic recovery falls in weight. The complexity of surgical or otherwise painful experimental procedures and the impact on research results make monitoring to a vital part of not only anaesthesia but also of scientific work itself. Moreover, from the view of animal welfare, it is an obligation to control laboratory animals after interventions in order to detect and treat any disease or impairment of their well-being.

However, in mice, the majority of classical monitoring systems are hardly applicable in the laboratory routine. One reason is, that many commercially available devices are not useful in such a small mammal, even when the mouse lays in anaesthesia (e.g. the normal heart rate values of up to 800 beats per minute in the mouse cannot be measured with common ECG devices, pulse oximetry sensors are to big to be placed on the mouse vessels). For the monitoring during the recovery phase, when the mouse is conscious and moves around, the measuring procedure would greatly influence animal's physiological parameters through restraint (e.g. ECG monitoring) or constant handling and manipulation (e.g. indirect blood pressure measurements).

2.8. Telemetry

Handling and restraining methods, especially those applicable for work with laboratory rodents, induce stress and influence basic physiological parameters. Those stimuli are so strong that they are actually used as models for the investigation of sympathetic and adrenomedullary systems [17, 18]. The responses catalogued to these stressors include changes in heart rate, blood pressure, blood flow, nociceptive threshold, as well as increases in plasma catecholamines, adrenocorticotropic hormone, prolactin, and corticosterone.

This situation has inspired the search for new technologies that would enable the monitoring of physiological parameters in freely moving animals while stress factors and artefacts caused by handling and immobilization are avoided. Telemetry represents a widely used in vivo technique in mice and other species for monitoring body temperature, heart rate, blood pressure and other variables in unrestrained animals.

Telemetric measuring eliminates the need for tethers that connect the animal with the data acquisition equipment. It also allows continuous data collection without disturbing the animal, and thereby presumably generating data that are not confounded by animal manipulation and associated physiologic effects [19-22].

The most common commercially available telemetry system is provided by Data Sciences International, St. Paul, MN, USA. This system is composed of implantable telemetry transmitters, receiver plates, receiver multiplexor or matrix which enables the use of up to 16 receivers simultaneously and a computer equipped with Dataquest PCI card for digitalizing and storing data for subsequent analysis.

The implantable transmitter suitable for use in small laboratory rodents (weight 3.6 grams, length 21.0 mm, width 9.1 mm, depth 11.4 mm, volume 1.9 cc) consists of a small cylindrical body with two coiled wires protruding from one end of the cylinder (Figure 4). The transmitter body contains an amplifier, a battery, radio-frequency electronics, and a magnetically activated switch which allows the device to be turned on and off. The surface of the transmitter body is covered with a silastic material, to prevent irritation of the visceral organs and subsequent inflammation and adhesions. The leads consist of flexible helically wound stainless-steel wires of 0.6 mm diameter. They are insulated with a silicone tube, which is red coloured for the positive and white coloured for the negative lead led (see also Figure 4).

In order to obtain a positive deflection from the analogue output of the receiver, it is recommended by the manufacturer, that the negative lead is positioned approximately at the right shoulder and the positive lead towards the lower left chest (Figure 3) [23, 24].

The transmitter passes the data to a receiver located beneath the animals' cage via a radio signal. The receiver transforms this into a digital signal. Digital information from the telemetry receiver is relayed to the data acquisition system via a multiplexer (analogue ECG adaptor) (Figure 8). The data acquisition system converts the raw data into an electrocardiogram and the numerical results of heart rate, core body temperature and locomotor activity counts. Finally, all data are stored on the hard disk of the computer for subsequent analysis.

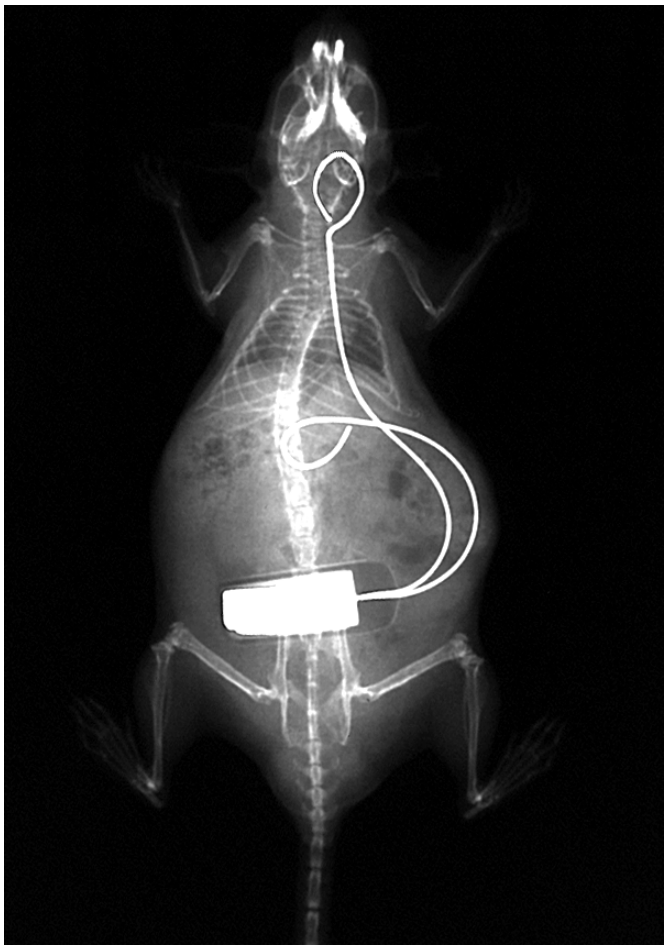

Figure 3 Radiograph showing location of the implanted telemetry transmitter. The body of the transmitter was positioned in the abdominal cavity. The positive lead was formed to a wire loop and fixed to the xiphoid process with sutures. The negative one was tunnelled subcutaneously from thorax to the neck and fixed as a wire loop between the muscles right and ventral of the trachea [25].

3. PROBLEM DESCRIPTION

3.1. Mouse anaesthesia: state of the art

Anaesthesia in rodents is common by injection of hypnotic, analgesic and muscle relaxant agents [2]. Since continuous intravenous, target-controlled infusion or the so called total intravenous anaesthesia with short acting drugs as propofol [26] is hardly to master in mice, the intraperitoneal or subcutaneous application route is normally chosen in this species [1, 27, 28]. It seems easy and practical, to induce general anaesthesia with a single injection of long-acting drugs (e.g. pentobarbital) or mixtures (e.g. ketamine/xylazine, medetomidine/midazolam/fentanyl), however, this type of anaesthesia is hardly controllable. Once the initial dose has been administered, the duration and depth of anaesthesia cannot be adjusted to the specifics (strain, age, gender, daytime, housing conditions, etc.) and individual reactions that influence the animals' response on the anaesthetic. Thus, despite previous dosage testing, the safety margin of injection anaesthesia regimens remains often narrow, i.e. anaesthesia is shallow in some individuals or the death rate can be unexpectedly high [1, 27, 29].

Such failures are rarely found with inhalation anaesthesia. The commercially available, modern volatile anaesthetics as isoflurane, sevoflurane, desflurane and others are vaporized in dedicated vaporizers and added to a carrier gas which is supplied to the animal via the respiratory tract. Due to their low blood:gas partition coefficients, they provide rapid induction of anaesthesia, are short-acting, and are removed in short time from the body, greatly by respiration [30, 31]. The dosage can be adapted easily and prompt to the individual animals' response. Thus, provided, that the animals vital functions and depth of anaesthesia are monitored, cases of death are unusual, because the course of anaesthesia is easy controllable and recovery seems fast. Due to their safety, recent volatile anaesthetics are increasingly used in laboratory rodents [2, 32], especially since ready to use inhalation anaesthesia devices, which are tailored for small rodents, are commercially available. The up to date anaesthesia equipments [33-35] normally include active systems for preventing the release of waste gas which is mandatory for protecting the personnel and which has been a problem in the past [36-38].

The most common and well-known volatile anaesthetic in laboratory rodents is isoflurane [2]. Sevoflurane, which is a more modern inhalation anaesthetic used primarily in human medicine [39] is less common in veterinary medicine due to its higher costs. Sevoflurane is suggested to have a weaker irritant action on the mucous membranes of the respiratory tract in man than isoflurane [40]. This common assumption was not confirmed by an investigation in rabbits, where it was found, that sevoflurane induced similar aversive behaviours of struggling and breath-holding as isoflurane when it

was used for the induction of anaesthesia by mask inhalation [41]. However, in rats and mice sevoflurane has been shown to induce less aversion than isoflurane [42].

From the other end, the possible action mode [43] and effects as neuroprotection [44] and cardioprotection [45] of these widely used inhalation anaesthetics are currently reported. However, the clinical impact of isoflurane and sevoflurane is described so far mostly in man or in animal species other than mice [46].

4. AIM OF THE STUDY

The continously increasing number of mice used in biomedical research, as well as development of complex surgical models and transgenic technologies, has brought anaesthesia in laboratory rodents under the spot light in the research community. Not only the questions of safety and feasibility, but also the changes of physiological parameters during anaesthesia and in the recovery phase fall in focus.

The goal of this study was to investigate possible advantageous properties and drawbacks of isoflurane and sevoflurane anaesthesia in laboratory mice. In a practical setting, the substances were compared by their effects during anaesthesia and in the post-anaesthetic recovery period from a clinical view, with the aim, to determine their impact on animal physiology and post-anaesthetic general condition in the laboratory routine. This will serve guidance for monitoring these types of anaesthesia and provide insight in their safety and in effects, which might interfere with results and aims of underlying biomedical experiments in laboratory mice.

5. MATERIALS AND METHODS

5.1. Animals and housing conditions

Sixty-four female C57BL/6J mice were obtained at the age of 6 weeks from our in-house breeding colony. The mice were free of all viral, bacterial, and parasitic pathogens listed in FELASA recommendations [47]. Their health status was monitored by a sentinel program throughout the experiments.

Animals were kept in type 3 open-top plastic cages (425 mm x 266 mm x 150 mm, floor area 820 cm^2) with autoclaved dust-free wooden bedding (80–90 g/cage). Autoclaved hay (8–12 g/cage) and 2 nestlets (each 5 x 5 cm), consisting of cotton fibres (Indulab, Gams Switzerland) were provided as nesting materials. A standard cardbox house (Ketchum Mainufacturing Inc., Brockville, Canada) served as shelter. Animals were fed a pelleted mouse diet (Kliba No. 3431, Provimi Kliba, Kaiseraugst, Switzerland) ad libitum and had unrestricted access to sterilized drinking water. The light/dark cycle in the room consisted of 12/12 h with artificial light (approx. 40 Lux in the cage) from 03.00 a.m. to 03.00 p.m. The temperature was 21±1°C, with a relative humidity of 50±5%, and with 15 complete changes of filtered air per hour (HEPA H 14 filter); the air pressure was controlled at 50 Pa.

Mice were housed in groups, except the 4 days before and 4 days after anaesthesia, where they were individually housed. The first day of single housing served for adaptation on the change of housing conditions; from the second day onwards the individuals' normal values for heart rate, core body temperature, locomotor activity, body weight, food and water consumption were taken. To avoid interfering influences, all necessary husbandry and management procedures were completed in the room before starting with single housing of mice and disturbances (e.g. visitors or unrelated experimental procedures) were not allowed. The animal room was insulated to prevent electronic noise.

The study was approved by the Cantonal Veterinary Office (Zurich, Switzerland) under the license number 111/2007. Housing and experimental procedures were in accordance with the Swiss animal protection law and conform to the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Council of Europe nr.123 Strasbourg 1985).

5.2. Preliminary transmitter implantation

Prior to the anaesthesia experiments, sixteen mice were instrumented at the age of ten weeks with telemetric transmitters. TA10ETA-F20 transmitters (Data Sciences International, St. Paul, MN, USA) able to measure heart rate, core body temperature

and locomotor activity in freely moving mice were implanted using the method established and described previously [23].

The day prior to the surgical implantation of the transmitter, a general health check was performed in these animals. Mice were weighted to ensure that none of the animals weight less than 20 grams, which in our experience represents the critical value for successful operation. Then, the hair from the belly and neck area was removed using a shearing machine.

The preparation of the telemetric transmitter and its implantation were carried out under aseptic conditions. On the day of implantation, firstly, the telemetric transmitters were removed from their sterile package. The leads were shortened and the sensory portions were prepared by removing the tubing and fixing the wires with silk sutures (6-0 Perma-Handseide, Ethicon, Norderstedt, Germany) to loops (Figure 4).

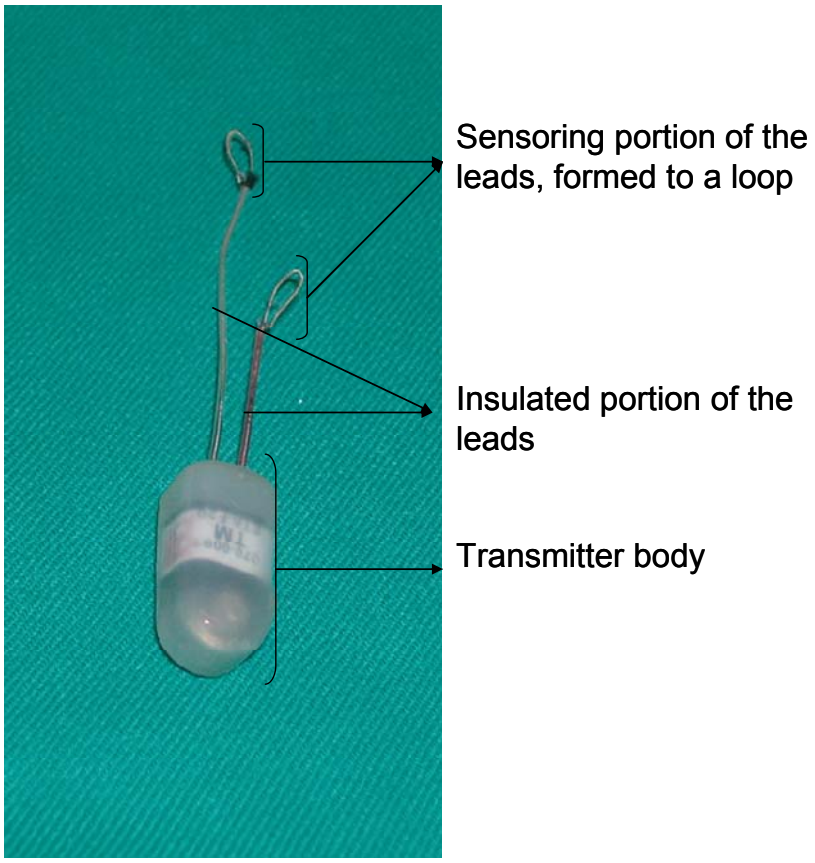

Figure 4 Picture of a TA10ETA-F20 implantable telemetry transmitter which collects heart rate, core body temperature and locomotor activity in the freely moving mouse. The sensing portions of the leads are free of silicone tubing and are formed to loops, which improves effectiveness and reliability of the signals as described previously [23]. The positive lead is indicated by the red coloured tubing whereas the white lead is the negative electrode.

Then, the mice were anesthetized by inhalation of the volatile anaesthetic sevoflurane (Sevorane™, Abbott, Baar, Switzerland) at a concentration of 3–8% in 100% oxygen at a flow rate of 400 ml/min. The anaesthetic gas was administered with a

nose mask. Ketamine (Ketasol-100™, Dr. Graub, Bern, Switzerland) was injected subcutaneously as pre-medication at a dosage of 45 mg/kg body weight as pre-emptive analgesia. To ensure maintenance of the core body temperature in physiological boundaries, mice laid in dorsal recumbence on a liquid-driven warming table set on 39 ± 1°C in the laminar flow of a work bench.

The skin of the neck and the belly was disinfected with iodine solution (Betadine, Mundipharma, Basel, Switzerland). A 1-1.5 cm long incision in the skin from the lower thorax to the abdomen along the midline was made. The negative lead was tunnelled subcutaneously from the thorax to the neck, where a small incision (0.5 cm) was made. The skin and underlying muscles were bluntly prepared to make place for the fixation of the wire loop. The electrode was fixed between the muscles located to the right of the trachea, using two silk sutures (6-0 Perma-Handseide, Ethicon, Norderstedt, Germany). The wound in the neck was closed then with absorbable sutures (6-0 Vicryl, Ethicon, Norderstedt, Germany) in layers. Then, the abdominal wall was opened and the telemetric transmitter body was implanted into the abdominal cavity of the mouse. The positive electrode was sutured to the xiphoid process with silk sutures (Figure 5). Finally, the muscle layers and the skin at the abdominal region were closed with absorbable sutures.

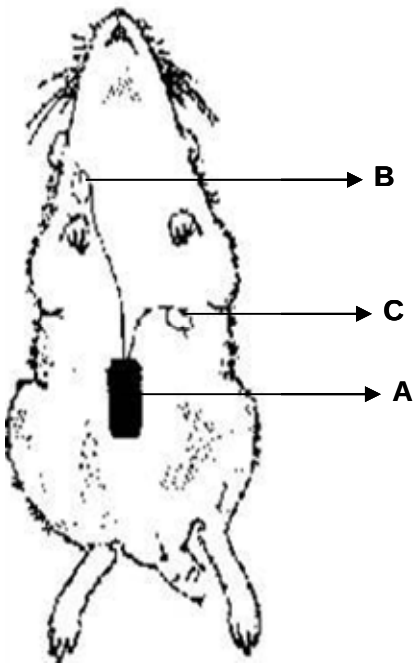

Figure 5 Localisation of the TA10ETA-F20 transmitter and position of the leads in the mouse. Such position is required to obtain a positive deflection on ECG with amplitudes large enough for subsequent analysis. A: The transmitter body is implanted in abdominal cavity. B: The negative lead is positioned between the muscles in the right neck area. C: The positive lead is fixed at the xyphoid.

Postoperative pain was treated with buprenorphine (Temgesic ™, Reckitt and Colman Products Ltd., Hull, England), at a dose of 0.1 mg/kg body weight, injected subcutaneously twice per day for 4 days. Animals were monitored daily for 10 days after the surgery for appearance, posture, spontaneous moving behaviour, body weight, and food and water consumption. After transmitter implantation, the mice had a period of 6 weeks convalescence until the start of the first anaesthesia experiment

5.3. General experimental setting

All experiments were conducted when the mice were aged 16--36 weeks, with body weights ranging from 24 to 30 g. To avoid any influence of circadian rhythm, all experiments and weighing procedures were carried out between 03.00 and 06.00 p.m.. Anaesthesia was performed in a separated operating area within the animal room to avoid transportation of the animals while ensuring stable conditions of humidity, air pressure and room temperature as well as air conditioning with sufficient extraction of gases and smells.

Anaesthesia was provided by a commercially available rodent inhalation anaesthesia apparatus (Provet, Lyssach, Switzerland), which was equipped with interchangeable vaporizers for isoflurane (Ohmeda Isotec 5, Abbott, Baar, Switzerland) and sevoflurane (Ohmeda Sevotec 5, Abbott, Baar, Switzerland). Waste anaesthetic gas was eliminated by a pump-driven filter system. As carrier gas, 100% oxygen was used in a flow rate of 400 mL/minute. The concentrations of anaesthetic gases in the induction chamber and nose mask were measured using a sidestream monitoring device which employs infrared technology (Datex-Ohmeda AS/3, Anandic Medical, Deissenhofen, Switzerland). The device was calibrated just before use using the proprietary standard reference gas of the producer company.

5.4. Determination of minimum alveolar concentration

Forty-eight, non-transmitter-implanted mice underwent anaesthesia 2-3 times in order to standardize anaesthesia by establishing minimum alveolar concentration. Care was taken that animals had a break of at least two weeks between the tests. Minimum alveolar concentration was determined with a protocol which was a modification of published methods [48-50].

Briefly, after inducing anaesthesia in the chamber for two minutes at maximum concentration of anaesthetic gases (5% and 8% for isoflurane and sevoflurane respectively), the mouse was taken out of the chamber and placed in dorsal recumbence on a liquid-driven warmed mat set on 39°C±1°C. Then, the anaesthetic gas was applied at defined concentration via a nose mask. After an equilibration time of 10 minutes, every 5 minutes during a total of 30 minutes, painful stimuli in form of pinching the tail, the toe or the interdigital webbing, the abdominal skin, and neck skin were ap-

plied. The reactions were evaluated as positive motor reaction or negative motor reaction to painful stimuli depending on whether the animal responded with any kind of movement.

Using this protocol, firstly rough estimation was done, within the concentration window (1%-3% and 2%-4% for isoflurane and sevoflurane respectively) in which we have empirically expected the values for minimum alveolar concentration to lay. Concentrations were graded in 0.25% steps and 10 animals per concentration were used.

Finally, 2 concentrations were picked, the lowest one at which 95% of animals had as negative and the highest one at which 95% had as positive evaluated motor reactions. Twenty-five animals per concentration were put through as verification of those concentrations being partial pressures bracketing the response or lack of response in our tested population. Minimum alveolar concentration was then calculated as a mean between the two.

5.5. Acid-base and blood gas analysis

Non-transmitter-implanted mice were used at 3-4 weeks after minimum alveolar concentration determination experiments, for obtaining arterial blood with the goal to assess the side effects of the anesthetics used on respiration and acid-base balance.

Arterial blood was taken under anesthesia at time points 10, 30, and 50 minutes of anaesthesia from 8 mice per anaesthetic and time point. Following incision of the anterior neck, the right common carotid artery was dissected. Using a fine-bladed pair of scissors, a small hole was cut in the artery and the arterial blood was collected in a heparinised syringe. Values for pH, pCO2, and HCO3 were determined immediately using a blood gas analyzer (AVL Compact 3, AVL List, Graz, Austria). The animals were euthanized immediately by cervical dislocation, before they could die from the subsequent rapid loss of blood under anaesthesia.

The normal values of pH, pCO_2, and HCO_3 used for comparison had been previously established from the arterial blood of 20 mice of similar age as those used in the present study [1].

5.6. Anaesthesia characterisation experiments

Anaesthesia was induced by setting the mouse in a clear perspex induction chamber (8x8x8 cm, volume 512 ml) in which then either 5% isoflurane (Isoflo™, Abbot, Baar, Switzerland) or 8% sevoflurane, carried by 400ml/min of pure oxygen, was introduced (Figure 6). Animals were observed for their behaviour to detect signs of excitation or distress due to the exposition to the anaesthetic atmosphere (e.g. trials to escape, breath holding, eye blinking, nose scratching, struggling). Mice lost the righting reflex in approximately one minute.

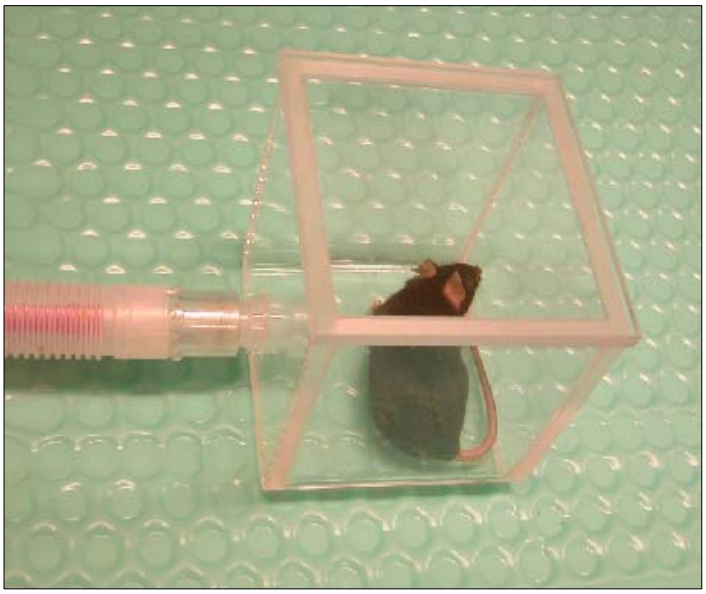

Figure 6 C57BL/6J mouse during induction. The chamber is placed on the water-driven warming mat.

After 2 minutes, the animal was quickly transferred to a nose mask, where anaesthesia was maintained with 2.8% isoflurane or 4.9% sevoflurane, equivalent to 1.5 X minimum alveolar concentration (the establishing of those values is described above). Mice were breathing spontaneously while they were laying in dorsal recumbence on a water-driven warming mat (Gaymar, TP500, Orchard Park, NY) set on 39°C±1°C to ensure constant body temperature (Figure 7).

Figure 7 Maintenance of anaesthesia with the nose mask. The mouse lay in dorsal recumbence on the warming mat for the whole time of anaesthesia.

The tail pinch, pedal withdrawal and abdominal skin pinch reflexes were proven to be negative in 5-minutes intervals and the respiratory rate was counted from the movement of the thorax wall.

Anaesthesia was stopped after 50 minutes by removing the nose from the mask and let the mouse breath room air. Two to three minutes later, when the mice had righted themselves from dorsal to ventral recumbence and were able to move, they were set back in their home cage.

5.6.1. Telemetric data acquisition and analysis

From sixteen transmitter-implanted mice, telemetric data were recorded in 8 mice per anaesthetic. Mice were allocated to groups in random order. Telemetric data were recorded with the Dataquest LabPRO program. Data collection was initiated by switching on the transmitter with a magnet. Data acquisition started 3 days before anaesthesia and continued for 4 days following the experiment. After data analysis was performed, all raw data were archived on institutes own server (ltkdco3-178-130).

For estimating acute effects of anesthesia (i.e. measuring data during the 50-minutes anaesthesia experiment), heart rate and core body temperature were recorded for 4 seconds every 15 seconds (four measuring points of 4 seconds per minute) while administering anesthesia. From these data, the mean values of heart rate and core body temperature were calculated for each minute in the individual. Normal values represent means from the time period 03.00-06.00 p.m. from three days prior to the experiment.

For investigating the post-anaesthetic effects of anaesthesia, heart rate and core body temperature were measured every 5 minutes for 30 seconds and 10 seconds, respectively. Locomotor activity was recorded continuously and stored at 5 minute intervals. Individuals' normal values were established by calculating the means from the three days prior to anaesthesia.

For the analysis of the long-term, post-anaesthetic effects, it was considered that values vary greatly during a 24h-cycle since mice are active mainly during the night. Therefore, the means of the telemetric values of an animal were calculated separately for the night and day phase. The difference between the night (12 h dark phase) and day (12 h light phase) values was calculated and compared to the individuals' normal values. This determination of circadian rhythms (delta night-day) of heart rate, core body temperature, and locomotor activity was adapted from an earlier description [51].

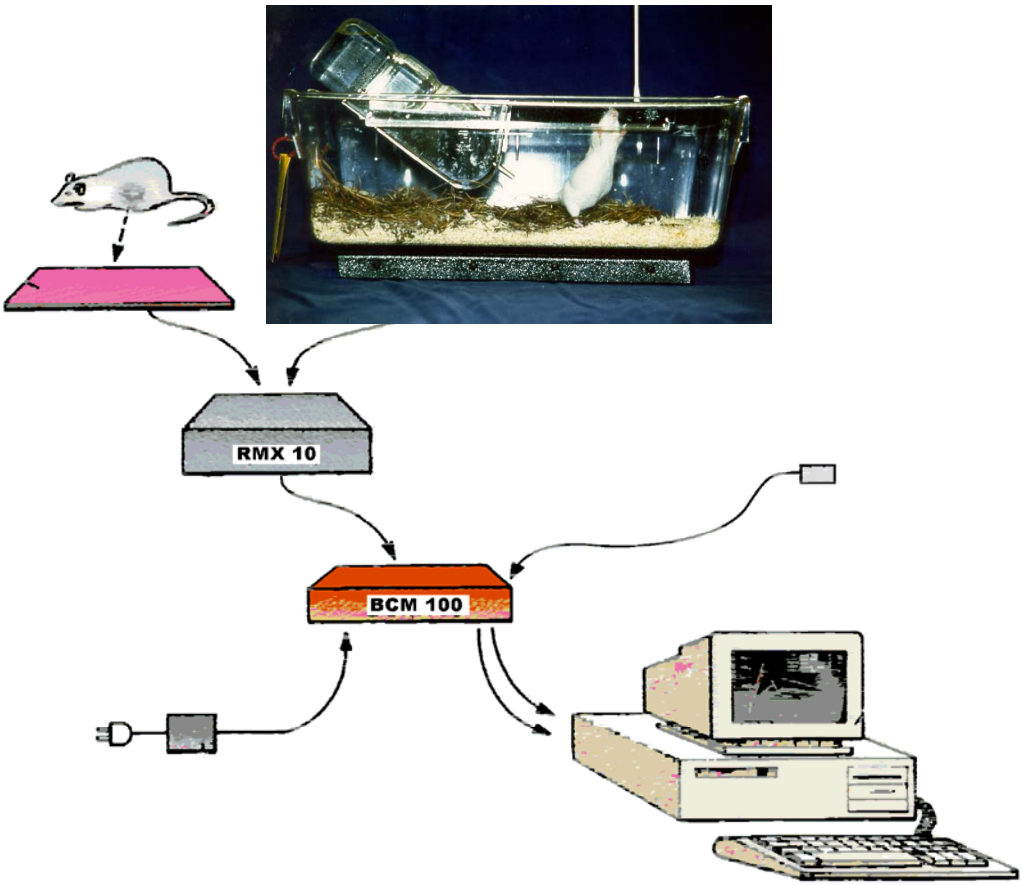

Figure 8 Schematic drawing of the telemetry acquisition system. The mouse is equipped with the transmitter, which measures the data and passes them via radio signals to the receiver plate. The receiver plate is located underneath the animal's cage. RMX 10 is a matrix which on the one hand provides power to the receivers and on the other multiplexes a number of signals into one that could be stored on the computer via the BCM 100 (Dataquest PCI card).

5.7. Monitoring of body weight, food and water consumption

The body weight progression and the daily food and water consumption were established from transmitter-implanted mice for 3 days before and 3 days after anaesthesia. Weights (animal, food pellets, water bottle) were recorded with a precision balance (PR 2003 Delta Range, Mettler-Toledo AG, Greifensee, Switzerland), especially adjusted for use with moving animals. Body weights recorded in transmitter-bearing mice were corrected to take into account the weight of the transmitter (3.6 g). The mean normal weights (from 3 consecutive daily measurements prior to the experiment) were calculated for each mouse, and compared to the weights recorded on each of the 3 days afterwards.

5.8. Statistical analysis

All data are presented as means with standard deviation. For statistical analysis, the program SPSS for Windows, version 13.0 was used. Heart rate, core body temperature and locomotor activity data were tested for normal distribution (Kolmogov - Smirnof test) and homogeneity of the variance (Levene's test). After passing these tests, a one way ANOVA was performed to compare group means of heart rate, core body temperature, locomotor activity and pH, HCO_3, pCO_2. Additionally, for the long-term telemetric results, post hoc comparison with Bonferroni tests was performed at each of the 4 days after anaesthesia with the corresponding normal values. P-values ≤ 0.05 were considered significant.

6. RESULTS

6.1. Minimum alveolar concentration

Using the methods previously described, we found 1.85 % and 3.25% to be the minimum alveolar concentration values for isoflurane and sevoflurane respectively in adult female C57BL/6 mice that we used in our experiments.

By providing anaesthetics in concentration of 1.5 X minimum alveolar concentration it is generally postulated, that 99.9% of animals do not react to painful stimuli [6, 7], i.e. that they should have reached surgical tolerance. Thus, all consecutive anaesthesia experiments were conducted with 2.8% isoflurane or 4.9% sevoflurane.

At this place it should be pointed out that minimum alveolar concentration values depend not only on age, sex and strain of the mice used but also on atmospheric pressure and temperature. Thus those values represent our laboratory specific results and should be taken more as guidelines rather than as absolute benchmarks.

6.2. Acute effects of anaesthesia

After animals were placed in the induction chamber (Figure 6) and exposed to the anaesthetic gas, they moved around but did not show any behaviour that would hint on excitation or distress. In particular, no obvious signs that would indicate irritancy from the anaesthetic atmosphere on the mucous membranes of the eye and the respiratory tract were observed (e.g., trials to escape, breath holding, eye blinking, nose scratching, struggling). All mice were immobilised, i.e. showed clear loss of righting reflex, within 1 minute after the anaesthetic has been introduced into the induction chamber.

Telemetric recordings revealed that putting the mouse in the induction chamber, resulted in tachycardia with 737.5±19.7 bpm for isoflurane and 750.7±22.4 bpm for the sevoflurane group (Figure 10). Normal values were established to be 523.0±39.4 bpm for the respective day time.

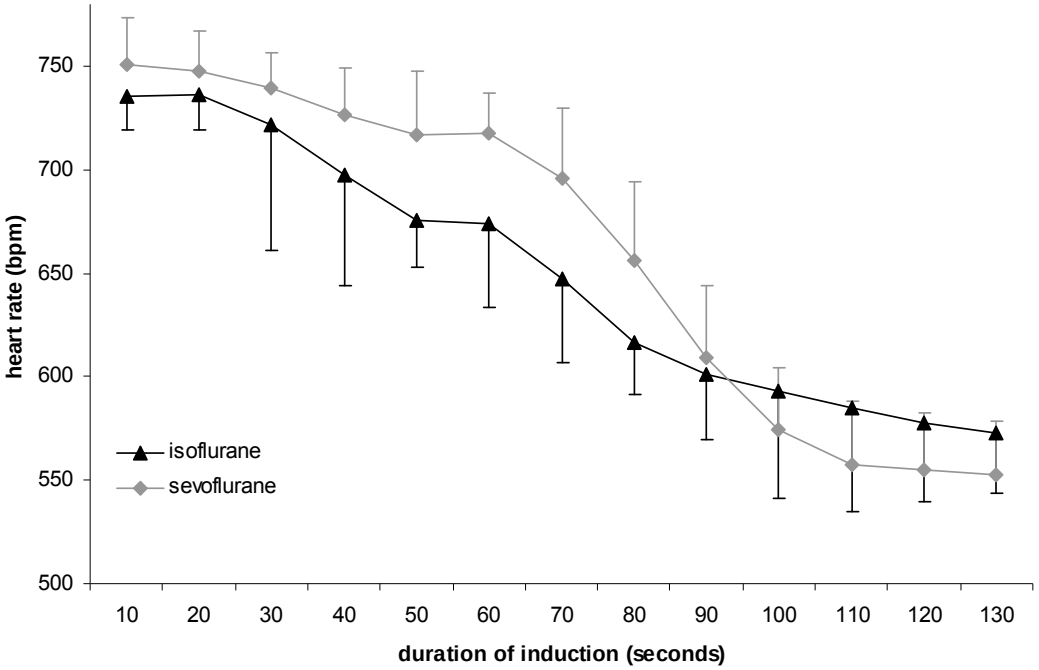

Figure 10 Heart rate during induction of anaesthesia with isoflurane and sevoflurane, n=10. Statistical analysis showed no difference between the treatments.

Analysis of heart rate during induction has also shown that approximately 100 sec after the introduction of the anaesthetic, the heart rate have reached the level at which it stayed stable during the whole time (50 minutes) of the anaesthesia. This was similar to the normal values obtained at the adequate day time in conscious mice before the experiment. Statistical comparison of the heart rate during the 120-seconds induction period showed no differences between the two treatment groups (Figure 10).

After induction, the mice were transferred on to the warming mat and the anaesthesia was maintained via a nose mask (Figure 7). Monitoring of heart rate, core body temperature and respiratory rate was maintained during 50 minutes of anaesthesia. Heart rate and core body temperature remained stable within the physiological boundaries. The respiratory rate was markedly decreased with 40.9±6.4 breaths/min and 44.4±5.8 breaths/min for isoflurane and sevoflurane respectively (mean of the 50-minutes anaesthesia). Thus, the respiratory rate was substantially below the normal values of the conscious mouse which was 150-170 breaths/min (Figure 11,12).

The lowest respiratory rate was observed after 2 minutes of induction, when it averaged 31.7±2.7 breaths per minute for isoflurane and 30.6±4.6 breaths per minute for the sevoflurane treated group respectively. Highest respiratory rates were noted after

10 minutes of anaesthesia with 54±7.8 and 50±9.7 breaths per minute for isoflurane and sevoflurane respectively (Figure 11).

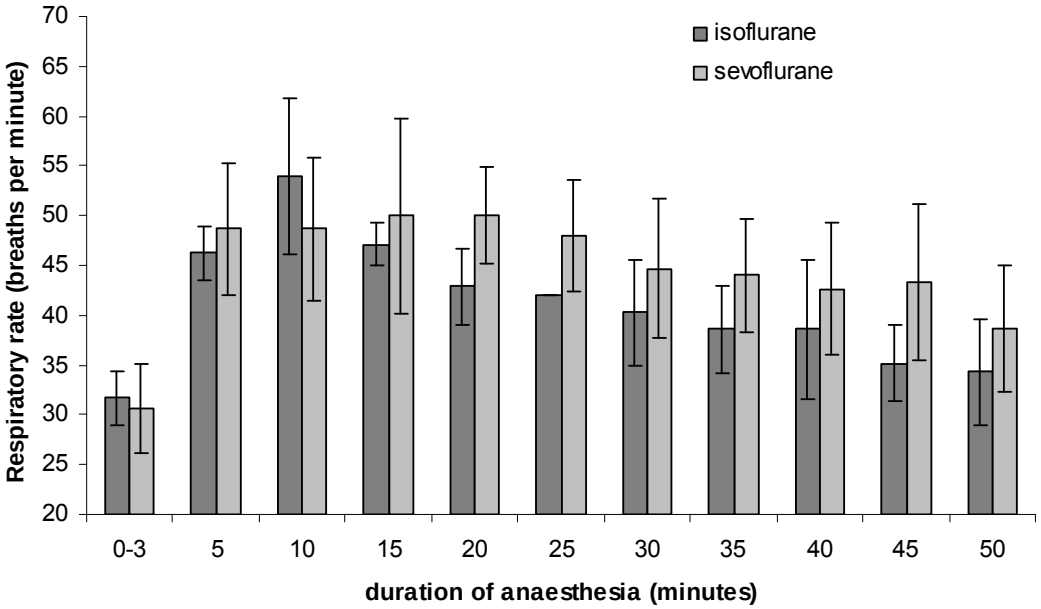

Figure 11 Respiratory rate during 50 minutes of anaesthesia with isoflurane or sevoflurane. Anaesthesia was induced in an induction chamber (time point 0-3 minutes) and was maintained via a nose mask (time point 5-50 minutes). Mice were breathing spontaneously. The respiratory rate was monitored by counting the movement of the thorax wall. Values were obtained and noted every 5 minutes. Mean values of 8 mice per group were depicted with bars representing standard deviations.

Acid-base balance and blood gas measurements revealed hypercapnia and subsequent respiratory acidosis at 10 to 50 minutes of anaesthesia (Figure 13). Already after 10 minutes, the pCO2 was increased. That trend continued during the subsequent measuring points, resulting in strongly increased pCO2 values (58.8±19.4 mmHg and 61.2±6.5 mmHg for isoflurane and sevoflurane respectively) after 50 minutes (Figure 13).Without being able to compensate in such a short time, pH slid into acidosis with 7.15±0.1 and 7.18±0.03 for isoflurane and sevoflurane respectively.

Statistical comparison of isoflurane and sevoflurane regarding all short term effects revealed no significant difference between both anaesthetics.

When anaesthesia was completed, animals showed increasing breath rate and muscle turgor. Within one minute, they turned in ventral position and moved at 1-2 minutes after anaesthesia was finished.

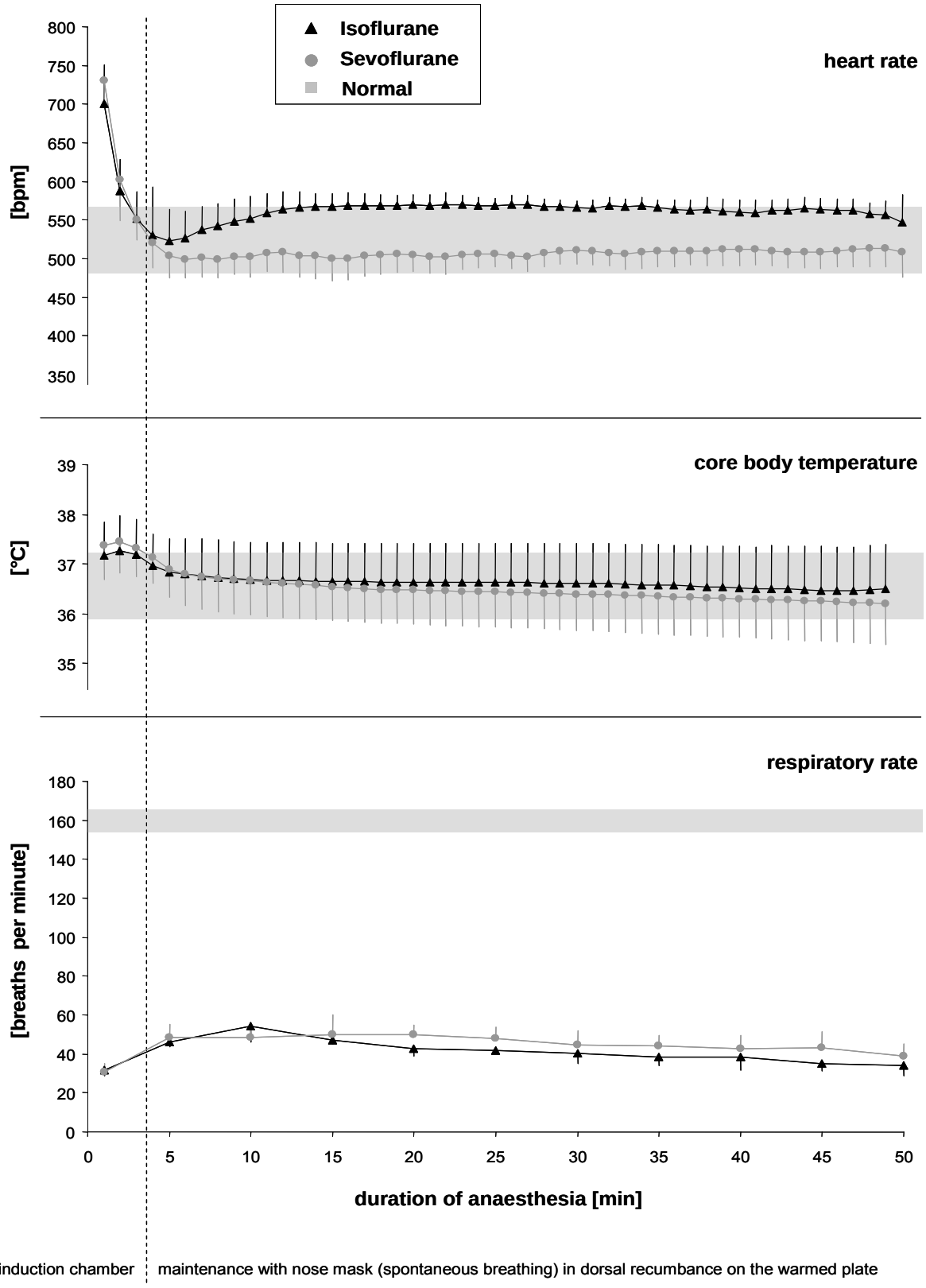

Figure 12 Heart rate, core body temperature and respiratory rate during 50 minutes of anaesthesia with isoflurane or sevoflurane. Anaesthesia was induced for 2 minutes in an induction chamber and then maintained with a nose mask. Animals breathed spontaneously while laying on a water-driven warming mat (39°C) to preserve core body temperature. The grey areas indicate range of normal values at the corresponding day time, when animals are conscious while mostly resting. Mean values of 8 mice are shown with bars representing standard deviation.

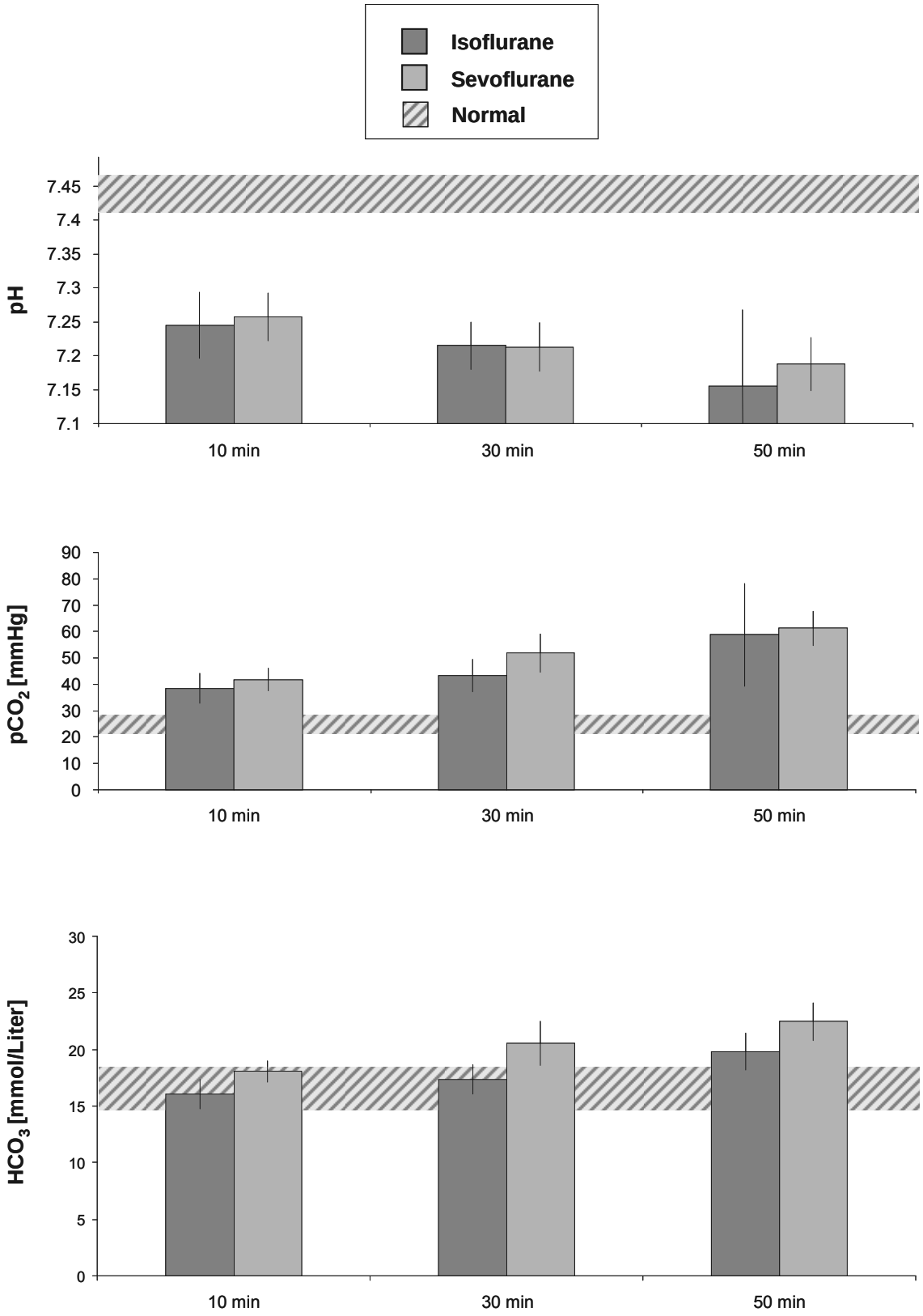

Figure 13 Analysis of acid-base balance (pH), partial pressure of carbon dioxide (pCO_2), and standard bicarbonate (HCO_3) in arterial blood show acidosis and hypercapnia. Hatched areas indicate range of normal values established in a previous study [1]. Mean values of 8 mice with bars for standard deviation are depicted. ANOVA revealed no difference between the impact of isoflurane and sevoflurane at any time point during anaesthesia.

6.3. Long term effects of anaesthesia

After animals awoke from anaesthesia, telemetric measurements of 8 mice in each group, over 4 days were performed. All animals served as their own control: delta values expressing the deviation from normal values established prior to anaesthesia were calculated for every individual and each parameter.

Mean delta values for heart rate and core body temperature showed significant (ANOVA; $p \leq 0.05$) increases during the first 12-hours daylight phase after anaesthesia. Mean delta heart rate reached values as high as 55.3±31.9 bpm over normal in isoflurane and 81.4±26.1 bpm over normal in the sevoflurane treated group. The increase in core body temperature were 0.6±0.36 °C and 0.5±0.18 °C for isoflurane and sevoflurane respectively (Figure 14).

Locomotor activity was slightly increased, but statistical analysis revealed no significant differences in comparison to normal (Figure 14).

Over the following period up to 4 days after the anaesthesia experiment, the telemetric recordings showed no statistically significant difference in none of the parameters measured in this study (Figure 14).

Body weight, food and water consumption were not changed at 1-3 days after anaesthesia (Figure 15).

Statistical comparison of isoflurane and sevoflurane regarding long-term effects revealed no difference between the two anaesthetics.

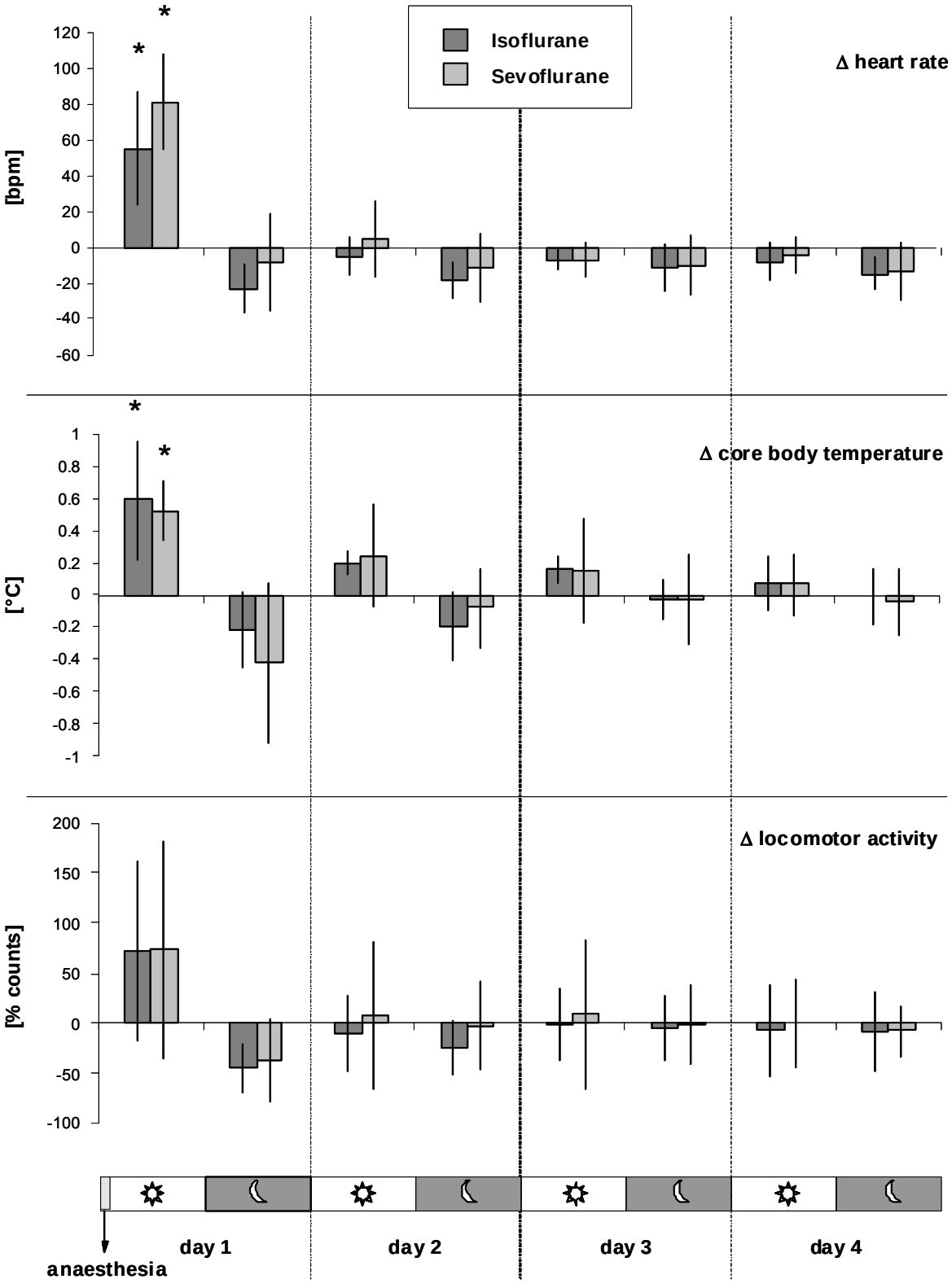

Figure 14 Long-term measurements of the impact of isoflurane and sevoflurane anaesthesia on heart rate, core body temperature and locomotor activity. Delta values represent the deviation from normal values (established prior to anaesthesia) at the corresponding 12-hours day and night time. The mean from 8 mice is depicted, with bars indicating standard deviation. Compared to normal levels, an elevation of heart rate and core body temperature was relevant at 12 hours after anaesthesia (ANOVA; * $p \leq 0.05$). No significant differences were found between the effects of isoflurane and sevoflurane.

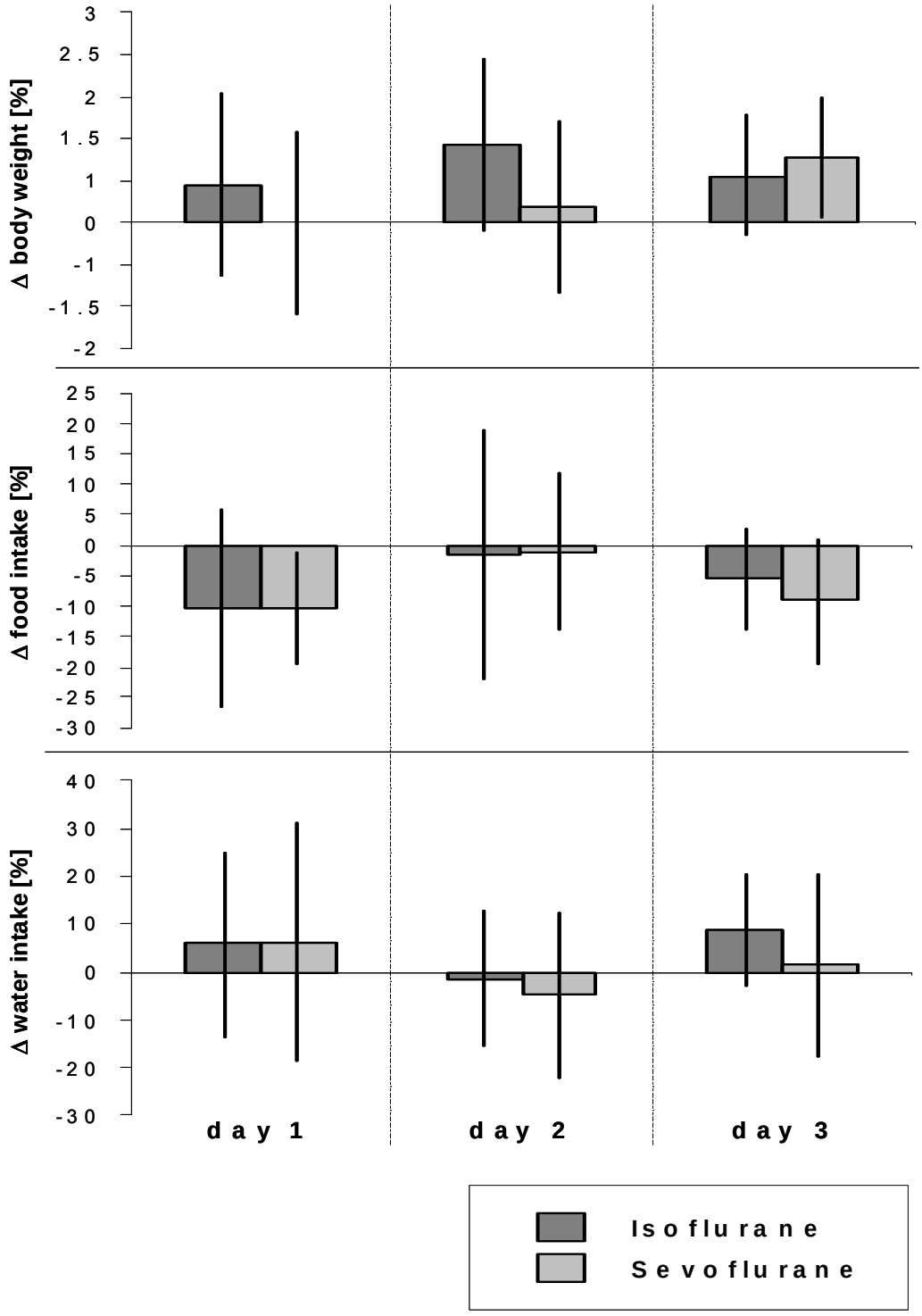

Figure 15 The percentage deviation (delta) from normal values of body weight, and food and water intake showed no relevant aberrations. Bars represent standard deviation.

7. DISCUSSION

In an easy to manage standard inhalation anaesthesia procedure, both volatile anaesthetics showed a high safety margin and short induction and recovery times. Particularly the induction of anaesthesia went smoothly without signs of excitation or either form of distress. During anaesthesia, the most prominent adverse effect was respiratory depression which hints to the respiration rate as a key indicator for monitoring and controlling depth of anaesthesia and thus preventing fatal outcome under routine conditions. Hypothermia, which usually occurs under anaesthesia, was successfully prevented by placing the animal on a warmed mat. After completion of anaesthesia, aberrations of physiological functions as elevated heart rate and core body temperature remained for approximately half a day. Thus, the post-anaesthetic impact of these inhalation anaesthesia methods is suggested to be of relatively short term relevance. Both anaesthetics showed almost identical properties and side effects, thus none could be proposed as superior over the other.

Before the investigations started the anaesthesia procedure and dosage of anaesthetics were standardized based on the specifics of the equipment (e.g., calibrating the gas concentration) and mouse population (e.g., strain, age, gender) used. Depth of anaesthesia was predetermined by establishing the minimum alveolar concentration with the aim to calculate the exact dosage for each anaesthetic, with which anaesthesia would be deep enough to allow surgical or otherwise painful interventions.

The minimum alveolar concentrations were established following the widely known method which is a tail pinch at that concentration of anaesthetic gas at which 50% of the animals respond with purposeful movements. In addition, we tested several reflexes in parallel to the tail pinch to find out, which other stimuli might be useful - or even more reliable than tail pinch - for defining the depth of anaesthesia. From the various stimuli, the pedal withdrawal reflex was found to correlate well with the tail pinch and other obvious signs of the different anaesthetic stages (e.g. presence or absence of spontaneous movements, vocalisation, muscle turgor and bodily tension). Altogether, the minimum alveolar concentrations were in the range described before for sevoflurane in outbred mice [52] and for isoflurane in C57BL/6J mice [4, 50].

Based on the preliminary testing, the anaesthetics were compared with dosages of isoflurane and sevoflurane that represented 1.5 minimum alveolar concentrations. For this dosage it is generally postulated, that 99.9% of animals do not react to painful stimuli [6, 7], i.e. that the animals have reached surgical tolerance. However, since analgesia was not proved with sophisticated methods as the measurements of the reaction of heart rate, blood pressure or respiration on a substantially painful stimulus (e.g. skin incision) surgical tolerance could not be claimed absolutely from our study.

However, with our regimens, all animals reached the desired stage of anaesthesia and none died, from which we conclude that the method was reliable and safety was high.

In the early induction phase of anaesthesia, heart rate peaked to the upper normal level of 700-800 bpm, which we suggest as a normal reaction on removing the animal from its cage and putting it in a foreign environment. During anaesthesia, heart rate ranged stable within the normal values of the resting mouse (490-550 bpm). This was according to data shown for isoflurane anaesthesia, that hint to only slight depression of heart and circulation resulting in intact hemodynamic conditions [32, 53-55]. Noteworthy, the cardiac depression from isoflurane was also less pronounced when compared directly with injection anaesthesia regimens [56, 57], which let the authors conclude that isoflurane is superior over injectable anaesthetics in those biomedical research studies, that focus on microcirculation or hemodynamic measures.

During anaesthesia, core body temperature was maintained with a simple, water-bath driven warming mat. The fall of core body temperature due to any kind of anaesthesia is well-known, e.g. a drop to 30-31°C was shown from isoflurane anaesthesia in mice [55]. Obviously, such hypothermia should be prevented, because it influences physiology and the course of anaesthesia and can finally lead to the dead of the animal. Thus, warming the animal in long-term anaesthesia with isoflurane is common practice since years and particularly worthwhile in mice because they are sensitive to hypothermia due to their small size and high body surface [54, 58, 59]. Thus, the warming was incorporated in our protocol and is advisable for safety reasons.

In contrast to the almost normal levels of heart rate and core body temperature, respiratory depression (Figure 12), documented by decreased respiration rates (far below the values of the resting mouse) (Figure 11), marked hypercapnia and acidosis are shown in equal grades from isoflurane as well as from sevoflurane anaesthesia (Figure 13). Hypercapnia and acidosis from isoflurane were also found by others [32], but were less intense when compared with injection anaesthesia with pentobarbital [57]. Respiration rate was also found to be markedly decreased from isoflurane by other studies, but was not as low reported from the literature as we have found it in both anaesthetics [58, 59].

Compared to other widely used protocols of laboratory mouse anaesthesia [1] isoflurane and sevoflurane provided a safe method of general anaesthesia (death rate 0), with virtually neglectable side effects upon heart rate and core body temperature. However, severe respiratory depression, caused by those two volatile anaesthetics, falls even more in weight when compared to results obtained from studies with injection anaesthesia (Figure 16).

In summary, respiratory depression was the major adverse effect in both isoflurane and sevoflurane (Figure 11, Figure 12) and might be the most probable emergency case to be observed when using this type of anaesthesia. From our opinion, it is rec-

ommended to monitor respiration rate closely when using inhalation anaesthesia. The respiration rate could hint on the depth of anaesthesia and could be useful, for adapting the dosing of the anaesthetic.

Time for induction and recovery were almost equal in both anaesthetics (1-2 minutes). As an advantage compared to injection anaesthesia, the short recovery time from both inhalational anaesthetics must be underscored. The fast recovery makes post-operative intensive care (e.g. after long-term anaesthesia or major surgery) much easier to manage because it allows detailed planning of work schedules and is less personnel intensive.

By using the telemetric technology, we could continuously collect real-time data over some days after anaesthesia without disturbing the animal. Thus, we managed to produce results that are not confounded by stress due to handling or restraining the animal. Therefore the results represent the actual impact of agents tested upon physiological homeostasis of laboratory mice. From this, we found a significant increase of heart rate and core body temperature for 12 hours after the animals had recovered from anaesthesia (Figure 14). Locomotor activity showed only a tendency to increase in the first hours after anaesthesia, indicating, that physical activity might be not the reason for the elevation of heart rate and core body temperature. Also it is remarkable, that other behavioural indices as food and water intake were not influenced by both anaesthetics (Figure 15), implying, that a 50-minutes inhalation anaesthesia with either isoflurane or sevoflurane are not impairing the overall well-being of laboratory mice.

Comparing isoflurane with sevoflurane, we found no relevant differences between both anaesthetics regarding their effects. Whereas several publications describe the usefulness of isoflurane, there is limited description of sevoflurane in mice in the literature. Henke and co-authors compared the induction and recovery times and respiration rate of sevoflurane with isoflurane in the gerbil, ending in the result, that there is no preference for one of the two volatile anaesthetics. They found prolonged recovery from isoflurane compared to sevoflurane and underlined warming of the animal as essential to prevent fatal hypothermia during inhalation anaesthesia [58]. Another study compared the blood glucose and some specific parameters required in functional PET investigations; they estimated sevoflurane superior over isoflurane and consequently recommended sevoflurane for physiologic imaging [60]. Others compared isoflurane with injection anaesthesia regimens in measures of blood glucose and found both anaesthetic regimens equally useful for performing the electroretinogram in mice [29]. Thus it is concluded that the side effects of both volatile anaesthetics, that may influence the outcome of a certain experiment must be defined under the specific experimental conditions and demands.

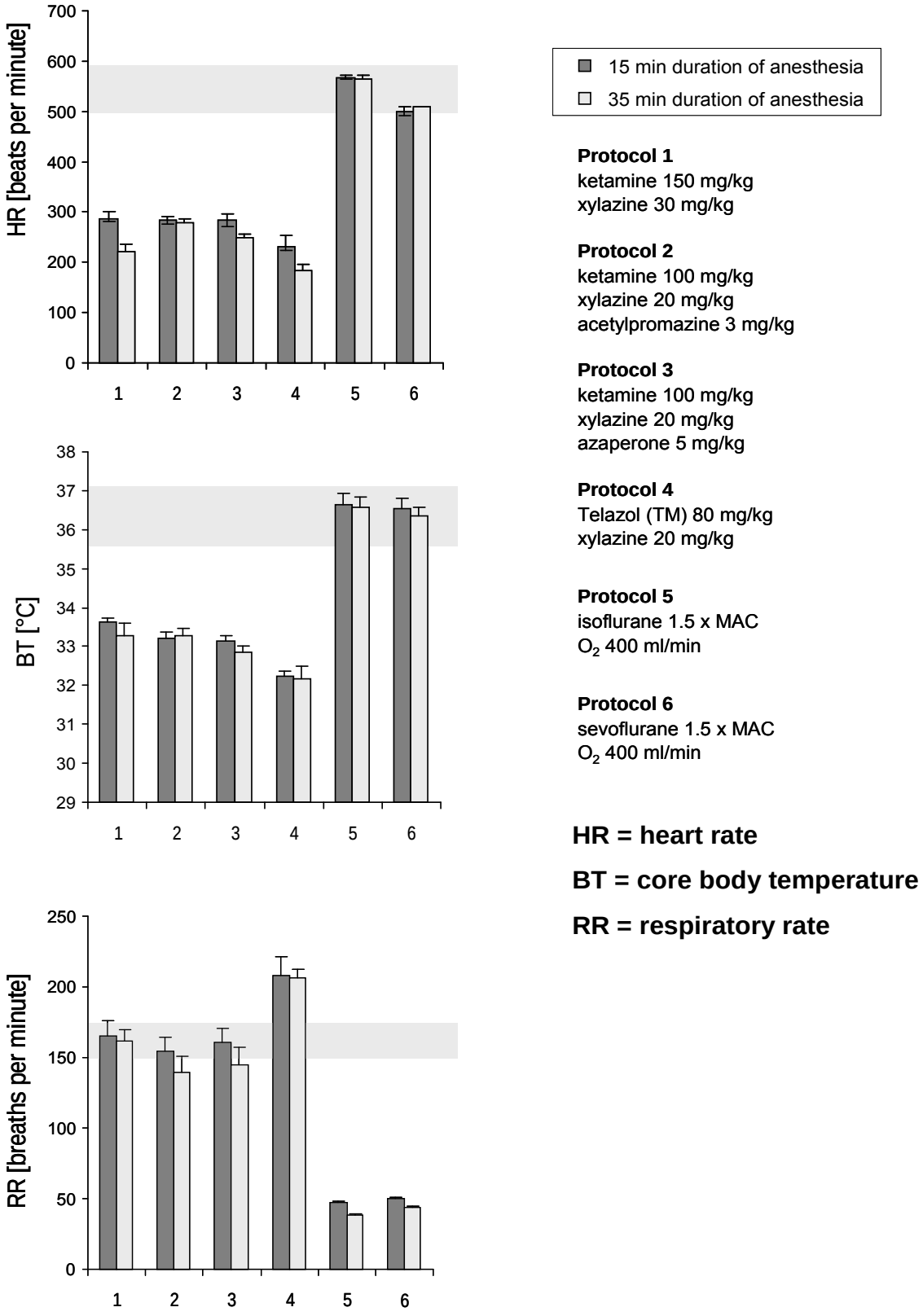

Figure 16 Comparison of injection anaesthesia vs. inhalation anaesthesia regarding their effects on heart rate, core body temperature and respiratory rate. Values for injection anaesthesia (protocol 1–4) were obtained from a previously published study [1]. Values for inhalation anaesthesia (protocol 5 and 6) represent data obtained from the present study. Grey background area represent range of normal values for conscious resting mice during corresponding day time.

In summary, we conducted inhalation anaesthesia in a routine, cost-effective setting, i.e. with the use of commercially available equipments as a simple anaesthesia machine with waste gas scavenge and a warming mat for preventing hypothermia. Anaesthesia was induced in an induction chamber and maintained with the mouse spontaneously breathing in a nose mask. As an alternative, it would be possible to perform the induction by restraining the mouse by the scruff of its neck and putting its nose in the mask. Thus, the mouse is – to our experience - immobilised within 10-20 seconds (own data, not shown). For checking the stage of anaesthesia, the tail pinch and pedal withdrawal reflexes are useful. Furthermore, the respiration rate might be helpful in monitoring the depth of anaesthesia and adjusting the dosage of anaesthetics to the individual needs of each animal during the progression of anaesthesia. Since respiratory depression was the most relevant side effect of anaesthesia, this adverse effect would be probably the reason if emergency cases (e.g. gasping, apnoea) or death occur. To avoid such fatal outcome, we suppose it advisable to take care, that the respiration rate is constant and will not drop below a certain level (e.g., 30 breaths per minute).

In conclusion, the described anaesthesia procedures would be feasible - or could be easily modified – to provide anaesthesia for a wide range of frequently used investigations and interventions in the laboratory routine. We performed an anaesthesia of 50 minutes duration, however, the time frame might be shortened or extended as desired. By use of such regimens, isoflurane and sevoflurane both provide a safe and reliable anaesthesia with short and smooth induction and recovery phases and relatively short acting, easy controllable side effects.

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